Study of Lipoprotein-associated Phospholipase A2 and Carotid Intima Media Thickness as Markers of Increase Cardiovascular Risk

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ABSTRACT

Introduction: Emerging risk markers that have demonstrated effectiveness in predicting risk for future cardiovascular disease events are carotid artery intima-media thickness (CIMT) and lipoprotein-associated phospholipase A_2. The aim of our study was to compare Lp-PLA_2 activity and CIMT in patients with risk factors of cardiovascular diseases.

Material and Methods: This case-control study included adult subjects with risk factors of cardiovascular diseases as cases and healthy age and gender matched subjects as controls. LP-PLA_2 activity was measured with ELISA method and CIMT measurements were made just before the bifurcation of common carotid artery by using B- mode ultrasound. Univariate and multivariate analysis was performed. A two-tailed P value <0.05 was considered statistically significant.

Results: A total of 70 cases and 70 controls were enrolled in this study. Multivariable stepwise regression analysis revealed glycosylated hemoglobin, and non-HDL cholesterol as independent predictors of CIMT. Lp-PLA_2 activity had no correlation with age, BMI, systolic blood pressure, diastolic blood pressure, lipid profile, serum uric acid, and CIMT.

Conclusion: The results from the present study reveal that Lp-PLA_2 activity increases with increment of number of cardiovascular risk factors. There was no relationship observed in predicting CIMT and Lp-PLA_2 activity

Keywords: Cardiovascular risk factors, Markers, Carotid intima media thickness, Lipoprotein-associated Phospholipase A_2

INTRODUCTION

Although prevalence of conventional risk factors such as smoking, hypertension, and hypercholesterolemia is not higher in South Asians than in other ethnic groups, yet, it is seen that some risk factors for atherosclerosis are particularly prevalent among them, including high triglyceride concentration, increased level of total cholesterol to high-density lipoprotein cholesterol ratio, type-2 diabetes mellitus and central or visceral obesity.[1-3] Two emerging risk markers that have demonstrated effectiveness in predicting risk for future cardiovascular disease events are carotid artery intima-media thickness (CIMT) and lipoprotein-associated phospholipase A_2 (Lp-PLA_2). It has been well established that CIMT is independently associated with CHD, and a consensus statement from the American Society of Echocardiography concludes that CIMT represents subclinical vascular disease, a marker of coronary heart disease (CHD).[4-7] Correlation between Lp-PLA_2 levels and traditional cardiovascular risk factors have been shown in several studies.[8-10] The aim of our study was to compare Lp-PLA_2 activity and CIMT in patients with risk factors of cardiovascular diseases.

MATERIAL AND METHODS

This case-control study was performed at our tertiary care centre from February 2014 to August 2015 after approval from ethics committee. This study included adults aged >40 years of either sex, attending department of general medicine at our tertiary care centre. 70 consecutive subjects with risk factors of cardiovascular diseases were selected as cases and equal number of healthy age and gender matched subjects were included as controls. Patients with history of any cardiovascular events were excluded from the study.

After taking the informed consent, a detailed history and physical examination was conducted. When possible, data were obtained directly from patients using the standardized data collection instruments as patient's proforma on lifestyle factors, current medication use, and medical history. Briefly, subjects were categorized into current smokers, ex-smokers, and never smokers and current alcohol consumers, ex-consumers, or non-consumers. When the patient was unable to provide answers, a proxy knowledgeable about the patient's history was interviewed.

These patients were than subjected to laboratory investigations including lipid profile, glycosylated hemoglobin, and Lp-PLA_2 activity. LP-PLA_2 activity was measured with ELISA method by Raybiotech. Inc. USA. The level of Lp-PLA_2 activity was expressed as nmol/min/ml.

CIMT measurements were made just before the bifurcation of common carotid artery by using B- mode ultrasound (carotid duplex scanning with a 7.5 MHz linear superficial array probe in B-mode). Maximal and mean CIMT were defined as the greatest and mean values, respectively, of CIMT measured from 3 contiguous sites at 1-cm intervals. Patients were then classified on the basis of a normal (<1 mm) or an abnormal (>=1 mm) CIMT. Coronary heart disease risk at 10 years in percent was calculated with the help of the Framingham Risk Score.

STATISTICAL ANALYSIS

The statistical analysis was done using SPSS (statistical Package for social sciences) Version 20.0 statistical analysis software. Parametric data have been presented as mean ± SD. Parametric data have been evaluated using Student "t"-test. Correlation coefficients were computed for the association of age, cardiovascular risk factors, and CIMT with Lp-PLA2.

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Multiple linear regression analysis was used to evaluate the contribution of independent factors. A two-tailed P value <0.05 was considered statistically significant.

RESULTS

A total of 70 cases and 70 controls were enrolled in this study and subsequently evaluated. Baseline characteristic of all cases and controls by gender are presented in table 1. The mean age (years) of the cases and controls was 54.80±8.09 and 55.12±12 respectively. Female to male ratio in cases and controls was 1:1.8 and 1:2.04 respectively.

Among 70 cases, the most common cardiovascular risk factor observed was diabetes mellitus in 30 (42.9%) cases, followed by Hypertension 26 (37.1%), Hyperuricemia 12 (17.1%), Hypercholesterolemia 11 (15.7%), and Obesity 4 (5.7%). History of Smoking and alcohol use was recorded in 51 (72.8%), and 53 (75.7%) cases.

The mean $LPPLA_2$ activity (nmol/min/ml) in cases and controls were 737.48±244.24 vs. 556.16±29.35 nmol/min/ml respectively. We observed that majority of the cases (64/70) were considered at elevated risk according to the >556.16 nmol/min/ml (mean $LpPLA_2$ in controls) cut point for $LPPLA_2$ activity. We also observed higher CIMT in cases as compared to the controls subjects. (0.74±0.18mm vs. 0.53±04mm, p <0.001) Comparison among groups showed no significant difference in Lp-PLA_2 and CIMT where cases with 3 or more risk factor were compared with those <3 risk factors, (752.71±265 vs.722.25±223, p< 0.60) (0.79+0.17 vs.0.73+0.11, P <0.059). Although not statistically significant, those with intermediate and high risk Framingham risk score (FRS) (i.e. >10) had higher mean LP PLA_2 activity and mean CIMT in comparison with Framingham risk score (FRS) of <10 (751.05±226.38 vs 723.91±222.98, p<0.77) and (0.76±0.18 vs 0.75±0.12, p<0.11). Univariate analysis of mean LP PLA_2 among subgroups: hypertensive, non hypertensive (OR=0.98; 95% CI 0.11–0.89; p<0.23); diabetics, non diabetics (OR=0.73; 95% CI 0.13–3.89; p<1.0); and smokers, non-smokers (OR=1.28; 95% CI 0.21–7.59; p<1.0) showed no significant correlation.

Multivariable stepwise regression analysis revealed glycosylated hemoglobin, and non-HDL cholesterol as independent predictors of CIMT (Table 2). Lp-PLA_2 activity had no correlation with age, BMI, systolic blood pressure, diastolic blood pressure, lipid profile, serum uric acid, and CIMT (Table-3).

DISCUSSION

In numerous studies, an independent association between Lp-PLA_2 concentrations and an increased risk of cardiovascular events has been observed in individuals with varying degrees of baseline risk.[11-14]

In the present study, we observed that the mean Lp-PLA_2 activity was higher in subjects with cardiovascular risk factors as compared to controls. There was a rise in Lp-PLA_2 activity with an increment of number of cardiovascular risk factors. This observation was in accordance to an earlier study in Asian population. Gong H et al observed that Lp-PLA_2 activity was significantly increased in Metabolic syndrome subgroups when compared with Controls and there was a linear rise in Lp-PLA_2 activity with an increment of number of metabolic syndrome components.[15]

variables	Case	Controls
Number	70	70
Age (years)	54±8	54±9
Sex (male/female)	45/25	46/23
BMI (kg/m^2)	23±3	22±2
SBP (mmHg)	140±12	120±9
DBP (mmHg)	99±10	72±7
HbA1c (%)	6.7±1.2	4.7±0.4
FBS (mg/dl)	148±81	96±18
PPBS (mg/dl)	204±99	104±9
Mean CIMT (mm)	0.76±0.15	0.53±0.04
Mean Lp-PLA_2 (nmol/min/ml)	737±224	556±29
Cholesterol (mg/dl)	179±43	123±14
Non-HDL (mg/dl)	135±44	72±15
HDL (mg/dl)	44±6	51±6
Triglycerides (mg/dl)	176±26	142±20
Uric acid (mg/dl)	6±1	5±1

Table-1: Baseline characteristics of cases and controls

Lp-PLA_2				
Variables	simple		multiple	
	r	p	ß	p
Age (years)	-0.025	0.84	-0.14	0.89
Sex	0.116	0.341	-1.1	0.276
BMI (kg/m2)	0.119	0.328	0.83	0.409
SBP (mmHg)	0.016	0.896	0.64	0.519
DBP (mmHg)	-0.034	0.778	-0.77	0.444
HbA1c (%)	-0.035	0.771	-0.88	0.378
FBS (mg/dl)	-0.087	0.474	-0.66	0.512
PPBS (mg/dl)	0.128	0.29	1.36	0.179
Cholesterol (mg/dl)	0.072	0.552	0.93	0.352
Non-HDL (mg/dl)	0.082	0.549	0.13	0.372
HDL (mg/dl)	-0.090	0.457	-0.43	0.666
Triglycerides (mg/dl)	-0.061	0.617	-1.91	0.06
S.Uric acid (mg/dl)	0.189	0.117	0.94	0.349
Mean CIMT (mm)	0.070	0.565	0.03	0.974
FRS	-0.057	0.639	-0.44	0.66

Table-2: Linear regression analysis of variables correlated with Lp-PLA_2 in cases

CIMT				
	simple		multiple	
	r	p	ß	p
Age (years)	0.92	0.44	0.057	0.679
Sex	0.136	0.26	0.236	0.144
BMI (kg/m2)	0.239	0.047*	0.06	0.634
SBP (mmHg)	-0.224	0.062	-0.026	0.826
DBP (mmHg)	0.355	0.003*	0.144	0.279
HbA1c (%)	0.473	0.001*	0.502	0.02*
FBS (mg/dl)	0.409	0.001*	0.174	0.598
PPBS (mg/dl)	-0.383	0.001*	-0.274	0.468
Cholesterol (mg/dl)	0.377	0.001*	0.747	0.436
Non-HDL (mg/dl)	0.368	0.002*	0.332	0.034*
HDL (mg/dl)	0.007	0.952	0.074	0.581
Triglycerides (mg/dl)	0.314	0.008*	0.115	0.409
Uric acid (mg/dl)	-0.028	0.816	-0.264	0.048*
Lp-PLA_2 (nmol/min/ml)	0.07	0.565	-0.03	0.788
FRS	-0.196	0.104	-0.217	0.145
*p<0.05				

Table-3: Linear regression analysis of variables correlated with CIMT in cases

In contrast to the previous studies, the current study found elevated CIMT in 11% (6) cases. Salonen et al. found that in 1,200 Finnish men (a population with high incidence morbidity and mortality from CHD), 20% had elevated CIMT.[16] The Rotterdam study, a prospective cohort study that examined over >7,000 men and women >55 years of age, reported 25% subjects had elevated CIMT.[17] In another study that utilized the 75th percentile criteria for elevated CIMT examined middle-aged (mean 47 years) firefighters. In a sample size of 50 subjects, 27 (54%) were shown to have elevated CIMT.[18] Reason for this is due to small sample size and difference in the cohort age in our study in comparison to these these studies.

The results of the present study revealed no relationship in predicting CIMT and Lp-PLA_2 activity. These findings support several clinical studies which suggested that premature coronary atherosclerosis as well as carotid intima-media thickness plasma was not influenced by Lp-PLA_2 activity. Campo et al. examined the relationship between CIMT and Lp-PLA_2 activity in 190 Sicilian middle-aged subjects and found no relationship between these measures.[19] Kiortsis et al. examined the relationship between CIMT and Lp-PLA_2 mass and activity in 100 subjects with known dyslipidemia. Like Campos et al they found no relation between CIMT and Lp-PLA_2.[20]

CONCLUSION

In conclusion, the results from the present study reveal that Lp-PLA_2 activity increases with increment of number of cardiovascular risk factors. There was no relationship observed in predicting CIMT and Lp-PLA_2 activity. The major limitation of this study was that Lp-PLA2 mass was not examined, which appears to demonstrate the more potent relationship. The results of this study are based on single measurements of circulating Lp-PLA_2, which may not reflect the true activity of Lp-PLA_2 over time in atherosclerotic plaques.[15]

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